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Kh. Naser El'-Din, and G.P. Svyatogor

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THE DEVELOPMENT OF TWINS FROM OVERRIPE ROE IN THE LOACH

By Kh. Naser El'-Din and G.P. Svyatogor

The features of the development of loach embryos from "overripe" /45* roe which has been in the female for a long time after ovulation were studied. The roe loses its capacity for fertilization with increasing overripeness, and the number of embryos dying increases. In many cases, development of twin forms is observed. Histological examination of twin forms revealed disturbances in their structure: absence of a notochord and changes in the morphogenesis of the neural tube and differentiation of the mesoderm.

Roe which has remained in the female for some time after ovulation is called "overripe". Such roe develops deformed and many of the egg cells are not fertilized. Development of two-headed forms and twins occurs in many cases. Thus, for example, Witschi /9/ observed the formation of twins from frog eggs fertilized 3-5 days after ovulation. Briggs /8/ noted the appearance of twins and triplets in 20% of embryos of amphibians developing from overripe roe.

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*The numbers in the right-hand margin indicate the corresponding pages in the original. - Transl.

L.M. Nusenbaum /3/ and V.M. Korovina /2/ observed that deformed, often two-headed forms develop from overripe roe of loach obtained at different times after ovulation.

We studied the development of twins from overripe roe of the loach (Misgurnus fossilis L.).

The experiments were done at the Faculty of Embryology of Leningrad University in May - June, 1979. The conditions in which the loach were maintained and mature egg cells were obtained in the laboratory by hypophysial injections, as well the methods of insemination, have been described in the literature /4/. In the absence of males, the females usually do not cast roe, but the experimenter can obtain roe when needed by pressing on the abdomen and sides of the female with his fingers.

Two series of experiments were done with overripe roe from 17 females. Series I included two experiments. The first experiment was done on two females: after obtaining the first control batch of roe, the females were placed in a tank with a water temperature of 15°, and roe was obtained for experiments 13, 20, 38 and 45 h after ovulation. A second experiment was done on 5 females: roe was obtained 24 and 45 h after ovulation. The experiments of series II were done on ten females. The water temperature in the tank was 19°. Roe was obtained 12 and 24 h after ovulation. Females of approximately the same mass were taken for each series. In each experiment, the roe was inseminated with sperm from the same male.

The experiments showed that as the roe becomes increasingly overripe, it loses its capacity for fertilization, the number of dying embryos increases, various deviations from normal development arise and in many cases twins develop. Cases of the development of twin forms were noted in 16 clutches of overripe roe from the 17 fish which we used.

The appearance of twins was noted in the development of roe obtained 13, 20, 24 and 38 h after ovulation from females which were maintained at a temperature of 15°, and 12 h after ovulation from females kept at a temperature of 19° (Table 1, 2, 3). Normal cleavage is disturbed in such roe; blastomeres of uneven size are formed; and the blastula is of irregular shape - the cells can be situated over a large area of the yolk ball; sometimes the formation of two blastodiscs, one of which can be very small, is observed.

In the experiments of series I, the roe taken 45 h after ovulation (at a temperature of 15°) completely lost its capacity for development (see Table 1, 2). In series II, roe taken 24 h after ovulation /46 (at a temperature of 19°) also failed to develop (see Table 3). The perivitelline space does not form in such roe and the egg cells in most cases become opaque. The degree of fertilization (in %) is greatly reduced, and all embryos which develop from such roe subsequently die.

TABLE 1

The results of the first experiment of series I with incubation of overripe roe of loach.

Срок перезревания, ч (1)	Степень оплодотворения, % (2)	Число, % (3): погибших зародышей (4)	близнецов (5)	уродливых зародышей (6)	Количество икринок в опыте (7)
Сразу после овуляции (8)	100	1	—	1	573
13	95	21	7	6	538
20	84	33	5	6	473
38	57	85	3	3	432
45	—	100	—	—	420

Key: 1 - overripe time, h; 2 - degree of fertilization, %; 3 - percentage; 4 - of dead embryos; 5 - of twins; 6 - of deformed embryos; 7 - number of eggs in experiment; 8 - immediately after ovulation.

Note. The table presents the results of an experiment with the roe of one female. No twins were found in the experiments on the other female.

TABLE 2

Results of the second experiment of series I with incubation of overripe roe of loach.

Срок перезревания, ч 1	№ самки 2	Число погибших зародышей 3	Число близнецов, % 4	Количество икринок, % 5
6 Сразу после овуляции	1	19	—	619
	2	111	1,2	371
	3	47	0,4	830
	4	16	0,9	371
	5	14	—	304
21	1	180	1,2	378
	2	449	15,2	579
	3	290	7,2	499
	4	189	10,9	413
	5	425	10,4	540
45	—	325	—	325

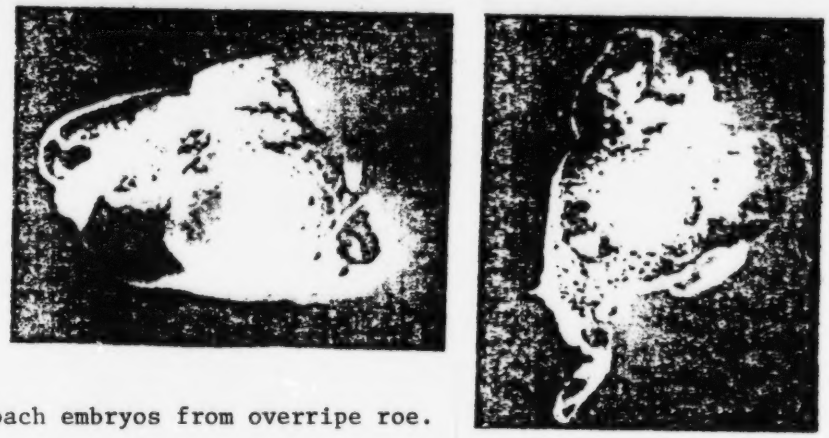
Key: 1 - overripe time, h; 2 - no. of female; 3 - number of dead embryos; 4 - percentage of twins; 5 - number of eggs; 6 - immediately after ovulation.

TABLE 3

Results of the experiments of series II with incubation of overripe roe of loach.

№ самки 1	Оплодотворение сразу после овуляции 2			Оплодотворение через 12 ч 3		
	число погибших зародышей, % 4	число близнецов, % 5	количество икринок в опыте 6	число погибших зародышей, % 4	число близнецов, % 5	количество икринок в опыте 6
1	7,7	—	376	42	4,3	1125
2	7,4	—	381	51	1,9	786
3	2,7	1	405	35	8,1	999
4	8,0	—	374	50	4,0	613
5	11,0	—	235	60	6,7	716
6	8,0	0,5	271	44	3,3	8 9
7	15,3	—	391	86	4,0	415
8	19,6	—	371	81	2,2	913
9	4,2	—	412	22	3,9	969
10	3,1	—	402	38	2,9	1204

Key: 1 - no. of female; 2 - fertilization immediately after ovulation; 3 - fertilization after 12 h; 4 - dead embryos, %; 5 - twins, %; 6 - number of eggs in experiment.



Development of double loach embryos from overripe roe.

The reduction in the capacity for fertilization in overripe roe, the death of embryos after fertilization, as well as the number of deformed embryos, depend not only on how long the roe has remained in females, but also on the temperature at which the females were maintained after ovulation until casting of roe. At high temperature, the capacity of /47 roe for fertilization is reduced, the number of embryos dying during the incubation of fertilized roe is increased considerably, and the number of deformed embryos is increased.

Most of the twins formed have been doubled at the anterior end. Sometimes they have a very small caudal part in common or they are joined at the middle of the trunk (Fig.).

The twins formed from overripe roe lag behind somewhat in development. At a time when normally developed larvae are emerging, some of the twins remain in the membrane for some time, and then hatch; others die inside the membranes. L.M. Nusenbaum /3/ considers this to be

the result of severe damage to the roe before fertilization and disturbance of the proteolytic enzyme processes associated with the physiological mechanisms of emergence of embryos from roe.

Histological study of twins obtained from overripe roe revealed various disturbances in their internal structure. The notochord may be absent in one or both of the twins; in addition, changes are observed in the morphogenesis of the neural tube and in the differentiation of the mesoderm.

Thus, with overripening of egg cells, various anomalies in development arise, a considerable portion of the embryos dies and cases of polyembryony are observed. L.M. Nusenbaum /3/ explains the disturbed development of overripe roe as the result of the asphyxia experienced by the egg when separated from the network of blood vessels after ovulation. Previously /7/, it was shown that the fertilized roe is most sensitive to asphyxia before the first cleavage; asphyxia leads to the appearance of deformed embryos. M.F. Vernidub /1/ found that pike roe consumes an increasing amount of oxygen as it ripens. The oxygen consumption of the ovulating cell is three times greater than before ovulation. An increase in the water temperature at which the females are maintained leads to accelerated ovulation and subsequently to accelerated manifestation of the effects of asphyxia after ovulation. V.M. Korovina /2/ explains the disturbance of normal development as a result of the suppression of the aerobic phase of respiration and compares it with the effects of asphyxia or low temperature.

Our observations allow us to state that with increasing overripeness

of roe, its ability to be fertilized is reduced and various deviations from normal development arise. Looking at our results from the perspective /48 of the theory of somatic embryogenesis /5,6/, we may conclude that overripening is an indication of a disturbance of embryo integrity, which later leads to the formation of two complexes of axial rudiments.

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